DNA CONTENT VARIATION AMONG HIGHER PLANTS[1]

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ABSTRACT

DNA content varies well over 100-fold among diploid herbaceous angiosperms. Differences exceeding two- to three-fold commonly exist among congeneric species. There is no overall correlation between DNA content and evolutionary or genetic complexity, and apparently only a small percentage of a plant genome has coding functions. The question of adaptive versus nonadaptive roles of variation in nuclear DNA content is addressed. The geographic, ecological, and taxonomic distributions of DNA content are not random; they apparently represent the results of natural selection. Several examples of evolutionary changes of DNA content and of geographical and ecological correlates are presented. Variation in DNA content is discussed in terms of the nucleotypic theory. The nucleotype is determined by the total quantity of DNA, both genetic and nongenetic, that has been correlated with several factors, including nuclear volume, cell volume, mitotic cycle time, and the duration of meiosis. It is proposed that differences in DNA content have striking and multiple nucleotypic effects on plant development and adaptation. A precise technique for the scanning microspectrophotometric determination of DNA content from Feulgen-stained leaf epidermal cells is presented.

Extensive variation in genomic DNA content exists among plant and animal taxa (Bachmann et al., 1972; Sparrow et al., 1972; Bennett & Smith, 1976; Price, 1976). Nuclear DNA amount among diploid herbaceous angiosperm species varies well over 100-fold (Bennett & Smith, 1976; Price, 1976). For example, *Arabidopsis thaliana* (2C DNA content = ca. 0.5 pg) and *Trillium erectum* (2C DNA content = ca. 80 pg) both have a diploid chromosome number of ten, but *T. erectum* has very much larger chromosomes housing about 160 times more DNA. Even more variation is apparent when polyploid species are considered, e.g., *Fritillaria davisii* (2C DNA content = ca. 225 pg) (Bennett & Smith, 1976). Differences in genomic DNA content exceeding two- to three-fold commonly occur among congeneric species, including those in the genera *Gossypium* (Edwards & Endrizzi, 1975), *Vicia* (Chooi, 1971), *Crepis* (Jones & Brown, 1976), and *Microseris* and *Agoseris* (Price & Bachmann, 1975).

Among eukaryotes, there is no strong correlation between DNA content and organismic or genetic complexity (Sparrow et al., 1972). As stated above, even closely related species vary greatly in DNA content. Furthermore, it has been estimated that only a small percent of the base pairs of DNA are utilized for coding proteins in plants (Flavell, 1980). The general lack of a correlation between organismic complexity and DNA content, the variation in genome size (DNA amount per genome) among closely related species, and the apparent surplus of DNA above that coding for proteins have been called the DNA C-value paradox (Thomas, 1971; Raff & Kaufman, 1983).

Differences in DNA content apparently involve redundant nucleotide sequences that are without coding function (Flavell et al., 1974). It is not the purpose of this paper to discuss the nature of these sequences. The reader is referred to Murray et al. (1981), Walbot & Goldberg (1979), and Flavell (1980, 1986) for discussions concerning repetitive sequences within plant genomes.

The role of genome size variation will be discussed in terms of phenotypic effects independent of any biochemical or coding functions that the nucleotide sequences may have. Topics included are (1) selfish DNA, (2) the nucleotype, and (3) taxonomic, geographical, and ecological distribution of DNA content variation. Lastly, a critique

[1] *Technical Article No. 22938 from the Texas Agricultural Experiment Station. Research supported by NSF grants DEB-7809940 and DEB-8009427. I thank Drs. Gary E. Hart and Ronald J. Newton for comments and constructive criticisms concerning the manuscript.*

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ANN. MISSOURI BOT. GARD. 75: 1248–1257. 1988.

of techniques for determining DNA content using scanning microspectrophotometry will be presented.

Selfish DNA

Orgel & Crick (1980) and Doolittle & Sapienza (1980) proposed that processes involved with DNA replication might allow the accumulation of DNA sequences in a genome whose presence stimulates the further accumulation of similar sequences. These sequences were considered to have no effect on the phenotype and to contribute little or nothing to the fitness of the organism. They suggested that this "selfish" or "parasitic" DNA may explain much of the variation in DNA content observed among species. These papers have resulted in an array of responses (see Nature 285: 617–620, 645–648, 1980) both favoring and criticizing the concept. In spite of controversy, the concept of selfish DNA is important because it points out the futility of searching for a biochemical function for all DNA sequences and because it stimulates thought on the origins and turnover of sequences in the genome. However, selfish DNA by definition excludes any functions for the sequences and thus diverts attention away from questions concerning evolutionary significance of variation in DNA content. If the generation of selfish DNA sequences is regarded as a mutational event that generates variation in genome size (Cavalier-Smith, 1980), then the important question becomes how much variation in DNA amount, regardless of mode of origin, is tolerated before selection comes into force.

The puzzle of genome size variation probably will not be solved at the subcellular level. Rather, the cellular effects of DNA amount are more likely reflected as a whole-organism phenomenon that can be best studied at the developmental, populational, and ecological levels. It has been proposed that the mere bulk of all the DNA in the genome exerts an influence on the phenotype so that under some circumstances selection favors accumulation of nuclear DNA more or less independently of its nucleotide sequence, while under different conditions the loss of sequences not necessary to survival may bring about considerable adaptive advantage (Bennett, 1972; Price, 1976; Bachmann et al., 1985).

Nucleotypic Effects

The nucleotype is defined as the effect of DNA quantity on the phenotype apart from the coding function (Bennett, 1972). The nucleotype is determined by the total quantity of DNA, both genetic and nongenetic, and influences several cellular and developmental parameters, including chromosome size, nuclear volume, cell volume, mitotic cycle time and the duration of meiosis.

General comparisons of karyotypes of plants with similar chromosome numbers showed that those with higher DNA contents have larger chromosomes (Sparrow & Evans, 1961; Sparrow et al., 1968; and others). Measurements of individual metaphase chromosomes indicated a strong positive correlation between DNA content, chromosome volume, and chromosome mass (see Rees & Jones, 1972; Levin & Funderburg, 1979). Furthermore, a strong correlation exists between the total length of the synaptonemal complex and genome size in plants (Anderson et al., 1985).

Baetcke et al. (1967) biochemically determined DNA content and measured nuclear volume from root meristems of 30 herbaceous angiosperms representing ten families. The DNA content varied over a 35-fold range among these species, and a linear relationship (slope = + 1) was demonstrated between DNA content and the mean nuclear volume of meristematic cells of root and shoot.

Price et al. (1973) measured cell and nuclear volume of apical meristem cells of species ranging over 100-fold in DNA content. The data indicated a direct correlation between nuclear volume, cell volume (slope = + 1), and DNA content. Additional data supporting a positive relationship between DNA content and cell volume include the research with unicellular algae by Holm-Hansen (1969), a study of a polyploid series of yeast, $1X$ to $8X$ (Gunge & Nakatomi, 1972), a correlation between the fresh weight and DNA content of root meristematic cells from thirteen angiosperm species (Martin, 1966), a relationship between DNA content and cell volume among *Gossypium* species (Edwards & Endrizzi, 1975), and an increase in cell size in rye with supernumerary chromosomes (Rees, 1972).

Nurse (1985) discussed the interaction of genes and DNA amount in the determination of cell volume. Although genes that influence cell volume have been studied, they cannot account for the correlation of cell size and DNA content. Factors other than DNA content that affect nuclear and cell volume of meristematic and differentiated cells include physiological differences, nutritional and environmental state of the plant, and the genotype (Price et al., 1973). However, in light of the strong correlation between DNA content, nuclear volume, and cell size, a reasonable hypothesis is to consider DNA content as determining a fundamental nuclear and cell size that is influenced by developmental, genetic, and environmental factors. Selection for

DNA amount may be partly through its effects on nuclear and/or cellular size.

The importance of cell size to plant adaptation needs much more research. Cutler et al. (1977) suggested that smaller cells help plants resist moisture stress because they maintain turgor with solute accumulation under lower water potential values than do larger cells. Nobel (1980) related water use efficiency (WUE = net rate of CO_2 uptake divided by net transpiration rate) in plant leaves to several factors, including diffusion across the cell wall and membranes, photochemistry, biochemistry, effects of the atmosphere, leaf temperature, stomatal opening, cellular properties, and leaf anatomy. The factor A^{mes}/A (mesophyll surface area per unit of leaf area) was mathematically modeled and experimentally demonstrated to affect WUE, i.e., an increase in A^{mes}/A results in an increase in WUE (Nobel, 1980). A^{mes}/A is strongly influenced by cell size, shape, and number, e.g., leaves of the same thickness but with smaller cells have a greater WUE (Nobel, 1980).

The effect of cell size on leaf physiology described by Nobel (1980) and others may be adaptive and related to the large changes in DNA content that have occurred during angiosperm evolution. Since cell size is positively correlated with DNA content, one major effect of varying DNA amount may be on traits related to WUE and osmotic adjustment. An evolutionary change for which decreased DNA amount might be adaptive is the ecological transition of a plant group from mesic to more xeric conditions. Individuals with less nuclear DNA may be better able to withstand water stress and higher temperatures due to the development of leaves with smaller cells, higher WUE, and turgor maintenance.

Many studies have established a relationship between nuclear DNA content and the minimum mitotic cycle time among diploid angiosperms (Van't Hof & Sparrow, 1963; Van't Hof, 1965; Price & Bachmann, 1976). Plants with higher DNA contents generally have a longer mitotic cycle. The average duration of the mitotic cycle increases at the rate of about 0.38 hr./pg of DNA (Evans & Rees, 1971). The increase is accounted for mainly in a longer period of DNA synthesis (Evans & Rees, 1971). An average difference of four hours in mitotic cycle time was shown between monocots and dicots of comparable DNA amounts; the difference is accounted for mainly in the increased length of the G_1 phase in dicots, which may be due to their more densely coiled chromatin (Evans & Rees, 1971; Evans et al., 1972).

The amount of DNA per nucleus also correlates with the duration of meiosis in diploid plants. The extremes reported are 18 hr. for *Petunia ×hybrida* (3C DNA content = 5.7 pg) and 274.0 hr. in *Trillium erectum* (3C DNA content = ca. 120 pg) (Bennett, 1971, 1977). The increase in the duration of meiosis with DNA content in these studies resulted from a similar increase in all meiotic stages.

The reasons for the DNA content differences among and within plant species remain matters of speculation. Certainly, the shorter mitotic cycle time and smaller cells may contribute to more rapid development of smaller annuals. However, additional factors should be considered. Mowforth et al. (1982) and Grime & Mowforth (1982) suggested that selection may operate on genome size through a differential effect of temperature upon cell division and cell expansion. They proposed that at low temperatures cell expansion is inhibited to a lesser degree than cell division. Therefore, growth under low temperatures was proposed to be promoted by cell enlargement, which would favor higher DNA content and larger cells. Under warmer temperatures, the advantage of growth dominated by cell enlargement should give way to that of growth involving higher rates of division of smaller cells with less DNA (Mowforth et al., 1982). Natural selection operating on such a phenomenon could have influenced both the inter- and intraspecific patterns of DNA content and ecological adaptation in higher plants.

Nucleotypic effects of variable DNA content are predictable and apparently of adaptive significance to higher plants (Price, 1976; Bennett, 1972; Bachmann et al., 1979). Evolutionary patterns of DNA content are discussed below.

Taxonomic, Geographic, and Ecological Distribution of DNA Content Variation

Geographic, taxonomic, and ecological distributions of genome size among herbaceous angiosperms are not random, but rather apparently represent the results of selection (Price, 1976). Karyotypic studies of grasses by Avdulov (1931) showed plants of tribes and genera centered in the tropics, or those that grow only during warm seasons in temperate climates, had uniformly small to medium-size chromosomes and nuclei. Plants of species growing in cooler temperature regions tend to have larger chromosomes. Levin & Funderburg (1979) concluded that genome sizes are generally larger in temperate compared with tropical herbs. However, in large cosmopolitan families other than the Gramineae and Liliaceae, no significant differ-

ences in genome size were detected between temperate and tropical species. It was suggested that generally families indigenous to tropical and subtropical regions have substantially smaller genomes than those of temperate regions.

Bennett (1976a, b) studied DNA content in relation to the distribution of cereal grain crops, cultivated pasture grasses, and legume pulse crops. Cultivation of species with higher DNA content tends to be localized in temperate latitudes, or the seasons and regions at lower latitudes where conditions are similar to those normally found in temperate latitudes. Bennett (1976a) suggested that man had generally chosen species for cultivation that resulted in a distribution paralleling or exaggerating the natural tropical–temperate cline in DNA content.

DNA content variation within genera is common. In cases where the phylogeny or primitive versus advanced status of taxa can reasonably be deduced, it is apparent that both evolutionary increases and decreases in DNA content have occurred. For example, Rees & Hazarika (1967) observed a three-fold variation in genome size among diploid species of *Lathyrus*. The higher values were from outbreeding perennial species and the lower from inbreeding annuals. This represented an apparent evolutionary decrease of DNA amount. Large evolutionary decreases in DNA content apparently have occurred in the evolution of annual species of *Crepis* (Jones & Brown, 1976), and in *Microseris* and *Agoseris* (Price & Bachmann, 1975). In contrast, the evolution of the inbreeding annual species of *Lolium* was accompanied or followed by an increase of about 35% in the quantity of nuclear DNA (Rees & Jones, 1967). The monotypic diploid perennial *Phalacroseris bolanderi* has more than twice the DNA of its perennial relatives of the Microseridinae. The higher DNA values probably represent an evolutionary increase (Price & Bachmann, 1975).

There is a general correlation of growth form with genome size. Bennett (1972) surveyed 271 plant species representing monocot and dicot annuals and perennials. His study showed that annual monocots and dicots have a significantly lower mean nuclear DNA content than perennials; the range of nuclear DNA amount is smaller among diploid annual species for both monocots and dicots; ephemeral annuals have a lower mean DNA content than nonephemeral annuals; and among monocots mean DNA content of obligate perennials is significantly greater than that of facultative perennials, and the mean values for facultative perennials and annuals are not significantly different. Bennett (1972) presented the hypothesis that nuclear DNA content and minimum generation time are correlated in plants, and that DNA content is causally correlated with the rate of development. He considered attributes that allow an annual species to develop rapidly in a time-limited environment (i.e., rapid mitotic cycle and brief meiosis) to require a low DNA content. Because of these nucleotypic correlates, plants with very high DNA contents should be perennials (Bennett, 1972).

DNA content has been considered in terms of evolutionary advancement within and among genera (Price, 1976) and growth forms of the species (Bennett, 1972), but it has received little attention within an ecological context. A better understanding of the apparent developmental and evolutionary significance of DNA amount comes from populational studies of diploid species that have been demonstrated to possess both interspecific and intraspecific variability in DNA content, such as western North American species in the subtribe Microseridinae (Asteraceae, Lactuceae). This group has been studied extensively by taxonomic (Stebbins, 1953; Chambers, 1955, 1963; Feuer & Tomb, 1977; Harborne, 1977), ecological (Chambers, 1955, 1957; Stebbins, 1972a), genetic (Chambers, 1955, 1963; Bachmann & Chambers, 1978, 1981; Bachmann & Price, 1979; Bachmann et al., 1979, 1981, 1983), and cytogenetic methods (Stebbins et al., 1953; Chambers, 1955, 1963). The genera *Agoseris* and *Microseris* are closely related; similar evolutionary trends have occurred within each. The perennial species of *Agoseris* and *Microseris* are taxonomically and ecologically primitive, and putative transition species morphologically bridge the gap between the genera (Chambers, 1957; Stebbins, 1972a, b). Trends toward reduction in size of all parts of the plant, more rapid growth rate, shortening of the life cycle, change of breeding structure from allogamy to autogamy, and specialization in structure of fruits and involucres have occurred in the evolution of annual species of both *Agoseris* and *Microseris* (Chambers, 1955, 1963; Stebbins, 1972a, b). The annuals have undergone a major ecological adaptive shift to drier ephemeral habitats and generally grow in late winter and early spring when moisture is available.

Relative nuclear DNA contents have been determined by scanning microphotometry of Feulgen-stained nuclei isolated from eight species of *Microseris*, four species of *Agoseris*, and *Phalacroseris bolanderi* (Price & Bachmann, 1975). A 7.7-fold range in DNA content was detected among these diploid ($2n = 18$) species. A 2.8- and 3.1-

fold range was found among species of *Microseris* and *Agoseris*, respectively. Price & Bachmann (1976) demonstrated a positive correlation between mitotic cycle time and nuclear DNA amount in the Microseridinae. Within *Agoseris* and *Microseris*, the annuals have lower DNA contents and a more rapid mitotic cycle than do the perennials.

Intraspecific variation in DNA content has been demonstrated in two annual species of *Microseris*. DNA content varies over 20% within *M. bigelovii* (Price et al., 1981a) and within *M. douglasii* (Price et al., 1980, 1981b). Within *M. bigelovii*, the lower DNA values were from geographically disjunct populations growing at the latitudinal extremes of the species. It was suggested that the small genomes may have resulted from selection for low DNA content in stressful and time-limited environments (Price et al., 1981a).

The DNA contents of 222 plants of *M. douglasii* representing 24 geographically, ecologically, and morphologically diverse populations in California were determined (Price et al., 1981b). The quantity was relatively uniform in most populations, even when there was an abundance of morphological diversity. Variation up to 14% existed among the population means. Populations with higher-DNA-content plants were restricted to more mesic sites, i.e., habitats receiving a yearly average precipitation of 20 inches or more, generally with well-developed soil. Price et al. (1981b) concluded that the observed distribution of DNA content in *M. douglasii* was not that expected by random drift; it was suggested that natural selection may have been responsible for at least part of the observed distribution pattern of DNA content.

The DNA contents of an additional 210 plants of *M. douglasii* were determined (Price et al., 1986). These data supplemented those previously reported (Price et al., 1981b) and allowed temporal changes in DNA content over several years to be detected at three populations. At one collection site near Jolon, California, temporal shifts were observed from low DNA amount in the drought year of 1962 to higher DNA values in 1973 after several years of generally more abundant precipitation, and back to low DNA content in 1977, the second of two severe drought years. These results suggested that the DNA content of *M. douglasii* may be tracking the environment and be subject to selection over seasons of drought and nondrought, respectively. Further collections (1980–1982) during the near-average to above-average rainfall years of 1978–1982 detected no reversal to a higher mean DNA content of the population. Although some high-DNA-content biotypes were detected by sampling for extremely robust growth forms, these had not become predominant over five years with very favorable moisture conditions.

A second population located near the summit of the Parkfield–Coalinga road had a mixture of very high, high, and low DNA biotypes in 1977 (Price et al., 1981b). The plants collected from 1980 to 1982 showed a progressive increase in mean population DNA content and a narrowing of the variation about the mean (Price et al., 1986). These results were compatible with the hypothesis that increased moisture availability and/or the longer growing season associated with it is conducive to selection for higher DNA values.

At a third population site near Middletown, mean DNA content was high in 1977. Since rainfall was low in 1977 in California, we expected to find after the high precipitation years of 1978–1982 a high mean DNA level in this population. Instead, the average DNA content of the samples declined between 1980 and 1982. This appears to contradict the hypothesis that increased soil moisture causes selection for higher DNA amounts. However, this site, a flat grassy swale, accumulates standing water after heavy winter rains. Factors such as the depth and persistence of standing water might affect the date of germination and the length of the time for maturation. Our field observations of late April to mid May in 1981 indicated that the plants of this population had a late start in growth, were depauperate, and were being exposed to a rapidly drying habitat during fruiting. Biotypes with lower DNA content might be favored at this site during years when winter rains produce a persistent vernal pool, which shortens the period favorable for growth and development.

The studies with *Microseris* suggest that one factor influencing the selection for DNA content is soil moisture availability. Other factors, such as length of the growing season, edaphic parameters, temperature, grazing, and competition from other plant species, apparently are of importance. The interactions of these parameters in influencing selection for DNA amount, however, appear to be complex, and DNA amount does not necessarily respond to selection in the same way at different sites if only one or a few factors are considered.

Another example of apparent adaptive intraspecific variation in DNA content is *Zea mays*. It has long been known that knob number in maize is negatively correlated with latitude in North America (Brown, 1949) and with altitude in Mexico (Wellhausen et al., 1952; Bennett, 1976b). Rayburn et al. (1985) and Laurie & Bennett (1985) independently determined nuclear DNA content from 21 and 10 lines, respectively, of maize representing a geographical range from Mexico to

Nova Scotia. DNA content varied up to 37%. A significant negative correlation ($r = -0.45$ and $r = -0.75$) exists between DNA content and latitude in both studies. Significant positive correlations existed between knob number (detected as mitotic C-bands) and the amount of karyotype consisting of C-bands ($r = 0.87$), knob number and DNA content ($r = 0.59$), as well as percent C-band heterochromatin and DNA content ($r = 0.70$) (Rayburn et al., 1985).

Corn is considered to have arisen in Mexico or Central America and then to have been taken northward by man. The selective pressures imposed in the northward migration of maize included a shorter and cooler growing season and maximum plant size permitted by climatic constraints (Rayburn et al., 1985). This selection for maximum plant size and rapid maturation appears to have involved reduction in DNA content through its nucleotypic effect of a shorter mitotic cycle time. The DNA primarily eliminated is apparently the 185-bp simple sequence satellite DNA that has been shown to be associated with chromosome knobs of maize (Peacock et al., 1981). Other possible effects of knob heterochromatin, such as influencing the expression of closely linked genes, could be of adaptive importance. Such phenomena remain to be detected at the molecular level.

Succinctly, nuclear DNA amount apparently has predictable and multiple nucleotypic effects on plants. Studies of development and physiology of closely related species or biotypes with different DNA contents, in relationship to their ecological adaptations and restraints, should lead to a better understanding of the evolutionary role of genome size.

Techniques—Determination of DNA Content

DNA content of nuclei from plant tissues is most commonly determined by scanning microspectrophotometry following Feulgen staining. Other methods include the biochemical extraction and measurement of DNA from an estimated number of cells (Martin, 1966; Baetcke et al., 1967), flow cytophotometry of isolated nuclei (Galbraith et al., 1983), and various nonscanning microspectrophotometric methods. Specific precautions for Feulgen staining and scanning microspectrophotometry will be discussed along with a specific protocol for DNA-content determination (Price et al., 1980).

SOURCES OF ERROR

Estimates of DNA content can vary among experiments due to many factors, including source of tissue, different preparation of fixative and stain, length of hydrolysis, temperature fluctuation, and adjustment and sensitivity of the microspectrophotometer.

The source of tissue is very important for DNA-content determination. Nuclei from similar tissues at the same stage of development from healthy plants are necessary if small differences in DNA content are to be accurately detected. It is not proper to compare interphase nuclei from one plant with mitotic nuclei of another.

The fixation of tissue is critical. Generally ice-cold 3:1 absolute ethyl alcohol and glacial acetic acid for 24 hr. provide adequate fixation. Some species or tissues may require a shorter fixation period. Standard and experimental tissue should be fixed with freshly prepared fixative from the same bottle. The material is transferred to cold 70% ethyl alcohol and stored in a refrigerator for up to approximately two months before staining.

One potential source of error is in the hydrolysis of tissue prior to Feulgen staining. Error due to hydrolysis can be minimized by performing a careful hydrolysis scheme and choosing a hydrolysis time that results in maximum staining of nuclear DNA. Too little hydrolysis will give reduced staining, and prolonged hydrolysis results in extraction of DNA and hence reduced staining. Therefore, the use of a hydrolysis curve can greatly reduce experimental error. Once an optimal hydrolysis period is established, it is important to hydrolyze together the standard tissue and the tissue being compared so that each receives a similar hydrolysis. Most species analyzed in my laboratory have maximum Feulgen staining following hydrolysis in 5 N HCl for 40–50 min. at 25° C.

Another source of error may result from different densities of nuclei. Polyploid cells and nuclei with high amounts of heterochromatin tend to have DNA amounts underestimated by Feulgen densitometry (Verma & Rees, 1974; Narayan & Rees, 1974). This problem can be overcome by measuring interphase nuclei rather than mitotic figures.

A frequently cited source of error is glare (Goldstein, 1970; Bedi & Goldstein, 1974). Error involving well-spread metaphase or telophase mitotic figures is potentially greater than that involving prophase or interphase nuclei (Bennett & Smith, 1976). Glare can be partially corrected electronically in the microspectrophotometer and by careful measurement of nuclei, including severe restriction of the area illuminated. In actual practice, stray light effects have been found to be negligible with the procedures using most modern microspectrophotometers.

After all sources of potential error have been minimized, measurements are then subject to sen-

sitivity of the microspectrophotometer. The assemblage initially used in my laboratory was a Zeiss Universal-II scanning microscope with a 03 photometer system, 0.5-μm scanning stage, modified 45-control unit, modified PMI-indicator, and a value average module. The latter three components have been recently replaced with a Zeiss Zonax, which contains a fully programmable set of controls and graphics system. Both systems allow for very accurate and precise measurement of DNA amount. The variance due to instrumentation in measurements accomplished with these systems is less than 1% of the mean.

In practice, the minimal differences in DNA content that can be detected by Feulgen microdensitometry are in the 2.0–5.0% range. To approach these, techniques must be used that minimize all sources of error, including slide-to-slide variation. This is achieved by the use of an internal standard on each slide (Dhillon et al., 1977; Price et al., 1980).

PROTOCOL FOR DNA CONTENT DETERMINATION

A technique for determination of DNA content from interphase leaf epidermal nuclei is outlined below. This technique leads to the highly controlled, internally standardized, and precise measurement of relative DNA content (Price et al., 1980) and has been successfully applied to several genera, including *Microseris*, *Agoseris*, *Coreopsis*, *Helianthus*, and *Zea*.

1. Peel epidermis from the middle third of nearly fully expanded healthy leaves of standard and experimental plants. It is best to use plants that have been grown in a growth chamber. The standard for *Microseris* is an inbred strain of *M. douglasii*.
2. Fix in ice-cold 3 : 1 absolute ethanol and glacial acetic acid for 24 hr., transfer to cold 70% ethanol, and store under refrigeration.
3. Place 5-mm squares of epidermis in a drop of H_2O on a cleaned (0.5 g gelatin, 1 g $CrK(SO_4)_2 \cdot 12\ H_2O$) microscope slide. Epidermis of the standard should be placed adjacent to that of the experimental.
4. Place coverslips over the epidermal squares with slight hand pressure and remove after freezing over dry ice. Floating slides over liquid nitrogen on a raft made of screen and styrofoam also works well and is more economical.
5. Air dry slides.
6. Hydrolyze for 40 min. in 5 N HCl at 25°C (hydrolysis time may vary in different species). Rinse in distilled H_2O.
7. Stain for 2 hr. in Schiff's reagent (see next section for preparation of Schiff's reagent).
8. Rinse for 10 min. in two separate SO_2 water (600 ml H_2O, 36 ml aqueous 1% $K_2S_2O_5$, 30 ml 1 N HCl) baths and 10 min. in H_2O. Blot away surplus H_2O with a paper towel.
9. Place a drop of enzyme solution (2% cellulysin (Calbiochem), 0.5% macerase (Calbiochem), 0.001 M EDTA, pH 5.6) over each epidermis for 30 min. (Feulgen staining is not affected by this treatment).
10. Dip slides gently into H_2O and absorb surplus water with a paper towel.
11. Place a cover glass on the tissue over a drop of 45% acetic acid. Apply moderate hand pressure. This releases nuclei from the cells and facilitates their movement to clear areas of the slide.
12. Remove cover glasses after freezing over dry ice or liquid nitrogen and air dry slides.
13. Mount No. 1 cover glasses over Permount. Let Permount harden for several days in the dark.
14. Spectrophotometrically measure (560 nm) DNA content of nuclei from the standard and experimental tissue on each slide. The values of individual nuclei of the experimental material on each slide are adjusted by multiplying the amount needed to increase or decrease the mean of the standard to a predetermined value. For the *M. douglasii* standard, it is 22.54 absorbancy units.
15. DNA values in picograms can be obtained by comparing the standard species with another species of known DNA amount.

PREPARATION OF SCHIFF'S REAGENT

Schiff's reagent quantitatively reacts with DNA during Feulgen staining and absorbs light at a peak wavelength of about 560 nm.

Prepare Schiff's reagent by dissolving 2 g basic fuchsin (Certified Biological Stain) in 400 ml boiling H_2O. After cooling, add 40 ml 1 N HCl and 4 g $K_2S_2O_5$. Store overnight in a dark stoppered bottle. Add 1 g decolorizing carbon, shake and vacuum-filter through No. 1 filter paper. The reagent should be used fresh; older reagent results in reduced staining of DNA.

ADVANTAGES OF EPIDERMAL PROCEDURE

The leaf epidermal technique with an internal standard on each slide has several advantages over

root tips or other cell types for microspectrophotometric determination of DNA content. For most composites, nearly all leaf nuclei are arrested at the G_1 phase of the cell cycle and are 2C in DNA content (Brossard, 1977; Nagl, 1978; Price et al., 1980). With *Zea mays*, 2C and 4C nuclei are readily identifiable. The epidermis from an inbred line of the same species used as an internal standard on each slide reduces errors due to variation in hydrolysis and staining from slide to slide and experiment to experiment. All values adjusted to the internal standard are directly comparable between slides and staining batches (Price et al., 1980). Spherically shaped interphase nuclei released by enzyme treatment are preferable to mitotic nuclei for microspectrophotometry, since hydrolysis and potential optical problems with compacted chromatin (Duijndam & van Duijn, 1975) are minimized. Lastly, the variance of interphase nuclei is among the lowest reported, with a CV generally under 5 or 6%.

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